(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34844 A1

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/US00/31005

(22) International Filing Date:
13 November 2000 (13.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/164,608 10 November 1999 (10.11.1999) US

(71) Applicant *(for all designated States except US)*: LIGOCHEM, INC. [US/US]; 1259 Route 46 East, Parsippany, NJ 07054 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: KRUPEY, John [US/US]; 175 Rutland Road, Glen Rock, NJ 07452 (US).

(74) Agent: SOLOMON, William, L; Antonelli, Terry, Stout & Kraus, LLP, Suite 1800, 1300 North Seventeenth Street, Arlington, VA 22209 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/34844 A1

(54) Title: METHOD FOR ISOLATING DNA FROM A PROTEINACEOUS MEDIUM AND KIT FOR PERFORMING METHOD

(57) Abstract: A method for isolating DNA from a proteinaceous medium such as whole blood, hemoglobin containing urine or saliva. Also disclosed are test kits for practicing the method.

TITLE

METHOD FOR ISOLATING DNA FROM A PROTEINACEOUS MEDIUM AND KIT FOR PERFORMING METHOD

FIELD OF THE INVENTION

The present invention relates to a method for isolating DNA from a proteinaceous medium such as whole blood, hemoglobin containing urine or saliva. Also disclosed are kits for practicing the method.

The method includes treating a specimen with a chaotropic agent such as aqueous guanidine thiocyanate containing a metal chelator such as ethylene diamine tetraacetate (EDTA); or alternatively, heating the specimen in the presence of guanidine thiocyanate without the metal chelator being present, adding a protein precipitating agent and isolating the liquid phase, treating this liquid phase with an adsorbent consisting of alumina, titania, or zirconia generated by flame hydrolysis in a solution of a metal salt which has weak charge attenuating properties, wherein the anion may be inorganic or organic.

BACKGROUND OF THE INVENTION

Nucleic acids are polymeric acids. In addition to having large numbers of nucleotides and ribose moieties, they possess a plurality of negatively charged phosphate groups. Because of their strong negative charge they should bind tightly to a positively charged metallic oxide surface. It has been demonstrated (Kummert R., and Strum W., International Journal of Colloid and Interface Science, 75(2) 373, 1980) that organic molecules with molecular masses smaller than 200 daltons and with the functional groups carboxylic, phenolic -OH or an amino group which can form covalent bonds with the structural metal, bind to the fumed aluminum oxide surface. The compounds that were employed in these studies were phthalic acid, benzoic acid, salicylic acid and catechol.

Since the primary focus is on the binding of polymeric acids to the oxide surface, very little is to be gained from the studies which employed monomeric molecules.

Further to the above, in general the binding of a polyelectrolyte (e.g. DNA) to a surface containing multiple permanent charges of opposite sign is energetically more favorable than the binding of a single isolated monomeric unit (e.g. a deoxy ribonucleoside triphosphate) to the same surface. The simultaneous presence of multiple interactions when the polyelectrolyte and surface are brought together may produce cooperativity between them, and together they might be much stronger than might be expected from the sum of their individual bond strengths.

In the case of single interactions involving the monomeric molecule and an oppositely charged surface, the single interactions are mutually exclusive or non-cooperative and hence the resulting bonds are relatively weak as compared to those between the polymer and the surface.

Boom et al, U.S. Patent No. 5,234,809 discloses a method for adsorbing nucleic acids onto silica particles in the presence of chaotropic agents. The silica-nucleic acid complex is then washed with organic solvents to prevent desorption of the nucleic acid from the solid phase. The nucleic acid is then eluted from silica using a mild buffer. There are fundamental differences in the chemistry and physical properties of silica and the fumed metallic oxides of the present invention.

Silica is an oxide of the element Silicon. Silicon has properties between metals and non-metals and is called a metalloid. Metallic oxides such as titanium oxide is an oxide of titanium which is a metal. A metal is a substance having a characteristic luster, malleability and high electrical conductivity, that is, metals readily loose electrons to form positive ions.

A metal can be thought of as an array of nuclei immersed

in a sea of electrons; some of the electrons present roam through the array of nuclei and acid and act as an all prevailing electrostatic glue. This is not the case with metalloids (silicon) where the electrons are less promiscuous and have a lesser tendency to wander about. All the atoms of metalloids are held together by a network of electron pair bonds. Substances with this type of structure are referred to as "network covalent solids". The entire crystal, in effect consists of one huge molecule.

When fumed titanium oxide of the present invention is placed in contact with water, its surface acquires a permanent positive charge. When this positively charged matrix is placed into contact with an aqueous solution of nucleic acid in either pure water, chaotropic salts or non-chaotropic salts (kosmotropes), a strong ionic bond is formed between the positively charged metallic surface and the negatively charged phosphate groups of the nucleic acid. The resulting nucleic acid-fumed titanium oxide complex is stable and cannot be dissociated by treatment with either pure water, alcohol, chaotropic ions or kosmotropic ions under neutral conditions. Dissociation is promoted by treatment with mild alkali.

When silica particles are placed in contact with water they do not acquire a permanent positive charge. Silica particles are mildly acid. Based on the experiments of Boom et al U.S. Patent No. 5,234,809, it appears that the interactive forces between the silica particles are weak in comparison to the strong electrostatic force that exists between the fumed metallic oxide and the nucleic acid since washing of the complex with pure water or neutral salt solutions tend to release significant amounts of nucleic acid from the surface. As a result of this property, Boom uses organic solvents to wash off extraneous proteins that are co-adsorbed onto the particles. Treating the nucleic acid-silica complex with an aqueous organic solvent to remove contaminating protein might be counterproductive, particularly

if the protein is insoluble in that solvent composition.

In order to release significant amounts of DNA from the nucleohistone complex of mammalian cells, the cells are treated with a solution containing a chaotrope. The accepted definition of a chaoptrope or chaotropic ion is a substance or anion which is least effective as a protein precipitant, and promotes unfolding, extension, and dissociation (Dandliker, W.B and de Saussure, V.A. in The Chemistry of Biosurfaces, Ed. M.L.Hair, Marcel Dekker, New York, 1971, p18). Examples of chaotropic anions are guanidine thiocyanate and potassium iodide.

At the opposite extremes are the kosmotropic ions. These substances are most effective as protein precipitants and lead to folding, coiling, and association. The helical content of the protein is thereby increased as a result of this treatment. Examples of kosmotropes are sodium chloride and sodium sulfate.

The process of protein destabilization is carried out in the presence of large amounts of chaotropes (3 molar to 10 molar for guanidine thiocyanate). At these concentrations, the extremely chaotic solution conditions overcome the molecular forces and cause destabilization of proteins. Boom et al employed a 10 molar solution of guanidine thiocyanate to displace the DNA from the starting material while a 3 molar solution of the same reagent was employed for dissociation purposes in the fumed metallic oxide procedure.

There is, however, a clear difference between the two methods with regard to the concentration of chaotrope that is employed during the adsorption process. The chaotrope requirements for the adsorption process of Boom, et al, U.S. Patent No. 5,234,809, are very stringent in that high concentrations of this reagent must be maintained to permit the adsorption of DNA to the silica particles.

In contrast, the chaotrope requirements for adsorption to

fumed metallic oxide surfaces are far less stringent, since the binding of DNA to this surface can occur at either high concentrations of chaotrope (5M) or at much lower concentrations of this reagent (0.01M) with equal efficiency.

U.S. Patent No. 5,057,426 discloses a method for separating long chain nucleic acids comprising fixing the nucleic acids onto a porous matrix, washing the porous matrix to separate the other substances from the long chain nucleic acids, and removing the fixed long chain nucleic acids from the porous matrix. The porous matrix is a material for chromatography having been modified with respect to its surface, and the material is based on a member selected from the group consisting of silica gel, diatomite, aluminum oxide, titanium oxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, and derivatives or copolymers thereof.

United States Patent No. 5,470,463 relates to modified porous solid supports and processes for the preparation and use of same. In particular, passivated porous mineral oxide supports are disclosed which are characterized by a reversible high sorptive capacity substantially unaccompanied by non-specific adsorption of or interaction with biomolecules. Passivation is achieved by use of a passivation mixture comprising a main monomer, a passivating monomer and a crosslinking agent, which mixture upon polymerization results in the substantial elimination of the undesirable non-specific interaction with biomolecules.

United States Patent No. 5,599,667 discloses the use of polycationic solid supports in the purification of nucleic acids from solutions containing contaminants. The nucleic acids non-covalently bind to the support without significant binding of contaminants permitting their separation from the contaminants. The bound nucleic acids can be recovered from the support. Also described is the use of the supports as a means to separate polynucleotides and hybrids thereof with a

nucleotide probe from unhybridized probe. Assays for target nucleotide sequences are described which employ this separation procedure.

United States Patent No. 5,635,405 discloses an aqueous colloidal dispersion for diagnostic or immunodiagnostic tests, comprising non-polymer nuclei surrounded by a hydrophilic copolymer that contains functional groups, a method for the detection of a specifically binding substance or immunochemically active component in a test fluid, and test kit containing the aqueous colloidal dispersion.

United States Patent No. 5,705,628 discloses a method of separating polynucleotides, such as DNA, RNA and PNA, from a solution containing polynucleotides by reversibly and non-specifically binding the polynucleotides to a solid surface, such as a magnetic microparticle, having a functional group-coated surface is disclosed. The salt and polyalkylene glycol concentration of the solution is adjusted to levels which result in polynucleotide binding to the magnetic microparticles. The magnetic microparticles with bound polynucleotides are separated from the solution and the polynucleotides are eluted from the magnetic microparticles.

There is a need in the art for improved methods for isolating DNA from a proteinaceous medium such as whole blood, hemoglobin containing urine or saliva. The present method overcomes the deficiencies of prior art methods noted above, and provides an improved method for isolating DNA from a proteinaceous medium.

SUMMARY OF THE INVENTION

The present invention provides a method for isolating DNA from a proteinaceous medium, such as whole blood, hemoglobin containing urine, or saliva, which comprises the steps of treating the specimen with a chaotropic agent, such as aqueous guanidine thiocyanate containing a metal chelator such as

ethylene diamine tetraacetate (EDTA); or alternatively heating the specimen in the presence of guanidine thiocyanate without the metal chelator being present, adding a protein precipitating agent and isolating the liquid phase, treating this liquid phase with an adsorbent consisting of alumina, titania, or zirconia generated by flame hydrolysis in a solution of a metal salt which has weak charge attenuating properties, wherein the anion may be inorganic or organic. Where it is inorganic a suitable anion is chloride, and where it is organic, acetate may be used. The procedure continues by separating the supernatant, washing the residue and removing the wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate, or phosphate or a metal hydroxide and recovering the liquid phase containing the DNA.

There is also provided a method for isolating DNA from whole blood by first lysing the specimen and recovering the nucleated cell fraction. The nucleated (white blood cells) are then treated with a chaotropic agent containing a chelator or treated with a chaotrope, and heated without the chelator being present. The sample is treated with a precipitating agent and the supernatant that is recovered is processed in the same manner as described above.

Alternatively, the white blood cell fraction may be treated with a surfactant (e.g. 1.0% aqueous sodium dodecyl sulfate, SDS) containing the metal chelator EDTA, adding potassium acetate to neutralize the SDS, and to precipitate hemoglobin and isolating the liquid phase, treating this liquid phase with an adsorbent consisting of alumina, titania, or zirconia generated by flame hydrolysis in a solution of a metal salt which has weak charge attenuating properties as described above. Further processing is achieved as described for the guanidine thiocyanate procedure.

There is also provided a method of isolating DNA from cells contained in a highly proteinaceous medium such as serum

or plasma, or from a medium which has very little or no protein such as urine, which comprises the steps of treating the medium with a chaotropic agent such as guanidine thiocyanate containing a metal chelator such as EDTA or alternatively, heating the specimen in the chaotropic agent in the absence of EDTA, and treating the liquid phase with either fumed alumina, fumed titania, or fumed zirconia followed by further processing as described. Alternatively, the proteinaceous or non proteinaceous medium may be treated with a surfactant containing the metal chelator and treating the liquid phase with the metallic oxide, followed by further processing.

There is also provided a method for isolating free DNA from a highly proteinaceous medium such as serum or plasma by adding fumed alumina, titania, or zirconia to the sample and processing the metallic oxide DNA complex as described above.

A method is described for the isolation of plasmids [e.g. Bacterial Artificial Chromosome (BAC)] from bacterial lysates which involve adding the protein precipitator ProCipitate™, followed by the addition of a fumed metallic oxide to the supernatant and processing the DNA metallic oxide complex to release free DNA as described previously.

A fumed metallic oxide configuration consisting of non attenuated charges was prepared by dispersing the particles in ion free water. This preparation had a high binding capacity for RNA and a marginal binding capacity for DNA and can be employed to remove contaminating RNA from DNA containing preparations.

The nucleic acid dispersed metallic oxide complexes generated are held together by strong ionic bonds contributed by multiply charged groups of opposite sign present on the respective interacting species. This situation presents a distinct advantage over the case that uses silica particles as adsorbent such as described in Boom, et al, supra. In the first place organic solvent need not be employed for washing

procedure.

Secondly, any buffer which contains an anion of unit negative charge such as a chloride, acetate and thiocyanate can be employed to thoroughly remove contaminating proteins from the dispersed metallic oxide complex without fear of desorbing the desired nucleic acid. Divalent anions such as sodium dodecyl sulfate may also be employed. Anions with a relatively high negative charge density such as phosphate, tetra-borate and citrate should not be employed.

The above and other objects of the invention will become readily apparent to those of skill in the relevant art from the following detailed description and figures, wherein only the preferred embodiments of the invention are shown and described, simply by way of illustration of the best mode of carrying out the invention. As is readily recognized the invention is capable of modifications within the skill of the relevant art without departing from the spirit and scope of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a diagramatic representation of charged fumed metallic oxide particles dispersed in ion free water.

Figure 2 shows a diagramatic representation of attenuated charges on fumed metallic oxide particles dispersed in a sodium chloride solution.

Figure 3 shows the aggregation and sedimentation profiles of fumed metallic oxides in the presence and absence of DNA.

Figure 4 shows the electrophoretic patterns of DNA isolated from whole mouse blood and form urine spiked with white blood cells using fumed titanium oxide P25.

Figure 5 shows polymerase chain reaction (PCR) patterns of DNA isolated form whole mouse blood and from urine spiked with white blood cells using fumed titanium oxide P25.

Figure 6 shows polymerase chain reaction (PCR) of low copy number DNA in plasma which was captured by fumed titanium oxide

P25 and subsequently released.

Figure 7 shows the electrophoretic profiles of the BAC DNA isolated by the fumed metallic oxide process and the alcohol-centrifugation procedure.

Figure 8 shows an example of the isolation of DNA using the method of the present invention in a 96 well format.

Figure 9 shows a schematic of the method of the present invention.

DESCRIPTION OF THE INVENTION

The present invention relates to a method for isolating DNA from a proteinaceous medium such as whole blood, hemoglobin containing urine or saliva. Also disclosed are kits for practicing the method.

The method includes treating a specimen with a chaotropic agent such as aqueous guanidine thiocyanate containing a metal chelator such as ethylene diamine tetraacetate (EDTA), or alternatively heating the specimen in the presence of guanidine thiocyanate without the metal chelator being present; adding a protein precipitating agent and isolating the liquid phase; treating this liquid phase with an adsorbent consisting of alumina, titania, or zirconia generated by flame hydrolysis in a solution of a metal salt which has weak charge attenuating properties, wherein the anion may be inorganic or organic. The description below further details the present invention.

Capture and Release of Nucleic Acids by fumed Metallic Oxides

A class of metal oxides produced by flame hydrolysis of the chlorides of the corresponding metals has been found to have broad-based applications in the fields of diagnostic and forensic medicine, as well as in the field of molecular bioinformatics. These reagents which are the highly dispersed metallic oxides of aluminum, titanium, and zirconium, show a high degree of specificity for the nucleic acids, DNA and RNA, present in aqueous media (blood, urine, saliva, waste water) and will bind

nucleic acid, irrespective of the volume of the sample.

Binding of either DNA or RNA to the metallic oxide surface occurs immediately upon mixing of the oxide with a solution containing the nucleic acid. Although binding of either DNA or RNA occurs immediately upon encounter with the metallic oxide surface, the geometric profiles of the two types of metallic oxide-nucleic acid complexes are radically different. The DNA metallic oxide complex is a white web-like structure consisting of a bridge work (i.e. a cross-linked structure) of DNA and metallic oxide. These aggregates settle rapidly under unit gravity.

The binding of RNA to the metallic oxide surface is less obvious, since no aggregation is visibly evident upon encounter. Nonetheless, an RNA-metallic oxide complex is formed, and is recovered after centrifugation.

The ability of the dispersed metallic oxides to search out and bind nucleic acids present in biological fluids is of great practical importance if it is desired to remove contaminating nucleic acids in the downstream processing of recombinant proteins. However, if this reagent is to have practical utility in the fields of forensics or diagnostics, the nucleic acid must be dissociated from the metallic oxide-nucleic acid complex in an intact form in order to permit its quantification and evaluation by current technologies (e.g. restriction enzyme digestion, polymerase chain reaction-PCR and sequencing).

The three highly dispersed oxides of aluminum, titanium and zirconium which are used in the method of the present invention are sold under the trademarks Aluminum Oxide C, Titanium oxide P-25 and VP Zirconium Oxide, respectively, and are manufactured by Degussa.

The basis of the process developed by Degussa is the hydrolysis of the gaseous metallic chlorides under the influence of the water, which develops during the oxyhydrogen reaction, and at the temperature characteristic for such a reaction.

The formation of the highly dispersed oxides takes place

schematically according to the equations:

Aluminum Oxide C

$$4AlCl_3 + 6H_2 + 3O_2 \rightarrow 2Al_2O_3 + 12HCl$$

Titanium Oxide P25

$$TiCL_4 + 2H_2 + O_2 \rightarrow TiO_2 + 4HCl$$

VP Zirconium Oxide

$$ZrCL_4 + 2H_2 + O_2 \rightarrow ZrO_2 + 4HCl$$

In principle, the corresponding highly dispersed oxide can be derived in this way from every vaporizable and hydrolyzable metal compound (Degussa, Technical Bulletin Pigments No. 56). The following compounds have already been produced in the laboratory or pilot plant:

Antimony (III) Oxide
Chromium (III) Oxide
Iron (III) Oxide
Vanadium (V) Oxide
Tungsten (VI) Oxide
Germanium (VI) Oxide

The nucleic acid binding properties of this list of fumed metallic oxides remains to be evaluated.

As a result of the flame hydrolysis process, the highly dispersed metallic oxides differ fundamentally from other oxides which are produced by precipitation, thermal decomposition or refinement of minerals. The dispersed metallic oxides of aluminum, titanium, and zirconium have an average particle size of 13 nanometers, 21 nanometers and 30 nanometers respectively and a specific surface of $100m^2/g$, $50m^2/g$, and $40m^2/g$ respectively.

The aluminum oxides produced through precipitation of aluminum hydroxide from aluminate solution followed by calcination consist of particles in the order of magnitude of micrometers. High surface aluminum oxide gels, in contrast to fumed aluminum oxide (Aluminum Oxide C) have a high proportion of internal surface.

In contrast to most solid microfine materials, the dispersed metallic oxides have the pronounced tendency to remain electrostatically neutral in the powder condition. However, when the dispersed metallic oxide, as exemplified by Aluminum Oxide C below, comes in contact with water, the substance of the solids binds protons and acquires a permanent positive net charge.

$$>AL\text{-}OH + H+ - AL\text{-}OH_2+$$

At the interface (boundary) of this positively charged species, the water molecules will experience orienting forces. The water dipoles will arrange themselves with their oxygen atoms facing the boundary, and their hydrogens facing into the aqueous phase as depicted in Fig 1. The dispersed metallic oxide surface becomes covered with a monomolecular layer of water with a net dipole orientation determined by the sign of the charge on the surface. Such pairs of separated charges across the boundary between two phases (in this case the metallic oxide and water) are called the electrical double layer or the electrified interface.

The Boom, et al patent is misleading, as it implies that a chaotrope, regardless of its concentration, whether it be high or low, is a suitable reagent for the dissociation of the nucleic and starting material and for the binding of the nucleic acid to the solid phase.

When present at lower concentrations (1.0-1.5 molar) a chaotrope may or may not exhibit chaotrope properties. It has been shown (Damodaran, S., Int. J. Biol. Macromol., 11,1, 1984) that chaotropes when present at a concentration in the range of 0.25-1.5 molar exert a stabilizing effect on bovine serum albumin. In other words, instead of causing dissociation and destabilization as would occur at high concentrations of chaotropes, they promote folding, coiling and association of the BSA molecule; an opposite effect. On the other hand, β lactoglobulin was destabilized under lower concentrations of chaotrope.

These observations contradict the general notion that the chaotropic effect of a substance on the stability of macromolecules is independent of any details of the macromolecular conformation itself. The data presented in this patent application suggests that the molecular and conformational predisposition of a protein may have certain influence on its structural destabilization or stabilization under low concentrations of chaotrope.

On the basis of these observations the concentration of chaotrope which was ineffective as a dissociating agent or which exhibits non chaotropic properties when employed for the adsorption of DNA onto fumed metallic oxide particles was determined.

Conducting a series of experiments using mouse blood treated with guanidine thiocyanate at concentrations ranging from 10 molar to 0.5 molar, it was found that no detectable amounts of DNA were released when the concentration of chaotrope was less than 1.5 molar. As a result, all adsorption experiments were performed at concentrations of chaotrope at 1.5M or less.

Behavior of nucleic acids toward fumed metallic oxides

The first series of binding experiments between nucleic acids (both DNA and RNA) and the dispersed metallic oxide surface were conducted in ion free water. The reagents were (a) calf thymus DNA (Boehringer Mannheim, Germany) at a concentration of 50 micrograms per milliliter in water, (b) RNA from calf liver (Sigma Chemical Co., St. Louis, Mo) at a concentration of 50 micrograms per milliliter in water, (c) dry fumed Aluminum Oxide C powder as supplied by the manufacturer, (d) dry fumed Titanium Oxide-P25 powder as supplied by the manufacturer, (e) a 2.5% weight/volume suspension of Aluminum Oxide C in water and (f) a 2.5% weight/volume suspension of Titanium Oxide-P25 in water.

Thirty eight milligrams of the respective metallic oxide

powders were added directly to one milliliter volumes of the respective nucleic acids. One and one half milliliter volumes of the metallic oxide suspensions were added to one milliliter volumes of the respective nucleic acids. All the tubes were mixed by inversion for about 10 seconds and then centrifuged at 10,000X g for 5 minutes. The supernatants were recovered and analyzed for the presence or absence of DNA and RNA by UV absorption at 260 and 280 nanometers. The results are reported in Table 1 below.

In another series of binding experiments the interaction between the nucleic acids and the fumed metallic oxide particles were conducted in an ionic medium. The reagents were (a) calf thymus DNA at a concentration of 50 milligrams per milliliter in a 0.15 molar sodium chloride solution, (b) RNA from calf liver at a concentration of 50 milligrams per milliliter in a 0.15 molar sodium chloride, (c) dry fumed Aluminum Oxide C powder, (d) dry fumed Titanium Oxide-P25 powder, (e) a 2.5% weight/volume suspension of Aluminum Oxide C in 0.15 molar sodium chloride and, (f) a 2.5% weight/volume suspension of Titanium Oxide-P25 in 0.15 molar sodium chloride solution. The pH of the respective nucleic acid solutions and the respective metallic oxide suspensions was adjusted to 7 by the addition of a few drops of a 0.1N sodium hydroxide solution.

Thirty-eight milligrams of the respective metallic oxide powders were added directly to one milliliter volumes of the respective nucleic acid solutions in sodium chloride. One and one half milliliter volumes of the metallic oxide suspensions in sodium chloride were added to one milliliter volumes of the respective nucleic acids in the ionic medium. The contents were processed as described above and the supernatants were analyzed for the presence or absence of the nucleic acids by UV absorption. The results are reported in Table 2.

The last series of binding experiments were conducted between fumed metallic oxide suspensions in ion free water and

the nucleic acids present in a salt containing medium.

For this purpose one and one half milliliter volumes of the respective metallic oxide suspensions in deionized water were added to one milliliter volumes of DNA and RNA respectively present in 0.15 molar sodium chloride. The mixtures were handled as described previously, and the nucleic acids recovered were measured spectrophotometrically. The results are reported in Table 3.

As shown in Table 1, very little binding (5.6-7.2%) of DNA to the respective metallic oxide particles took place when the interaction was conducted in ion free water. On the other hand, when the respective powders of the oxides were added to the DNA present in ion free water, there was a dramatic increase (greater than 90%) in DNA binding.

The binding of RNA to the respective metallic oxide surfaces in an ion free medium was found to be greater than 90% on the particles that had been added in a dry form as well as for the oxide suspension in ion free water.

Table 1

Binding of DNA and RNA in deionized water to fumed metallic oxides in deionized water

Nucleic acid in ion free water	% bound to Aluminum oxide C added as a dry powder	% bound to Aluminum oxide C added as a suspension in ion free water	%bound to Titanium oxide P25 added a s a dry powder	% bound to Titanium oxide P25 added as a suspension in ion free water
DNA	93.4	7.2	92.3	5.6
RNA	92.6	93.4	94.7	94.5

Table 2

Binding of DNA and RNA in salt solutions to fumed metallic oxides in salt solutions.

Nucleic acid in 0.15M NaCl	% bound to Aluminum oxide C added as a dry powder	% bound to Aluminum oxide C added as a suspension in 0.15M NaCl	%bound to Titanium oxide P25 added a s a dry powder	% bound to Titanium oxide P25 added as a suspension in 0.15M NaCl
DNA	90.1	91.3	92.4	91.8
RNA	94.2	92.6	92.4	90.6

Table 3

Binding of DNA and RNA in salt solutions to fumed metallic oxides in deionized water.

Nucleic acid in 0.15M NaCl	% bound to Aluminum oxide C added as a suspension in ion free water	% bound to Titanium oxide P25 added as a suspension in ion free water
DNA	6.7	5.2
RNA	91.2	92.4

It is evident from Table 2 that the DNA binding properties of metallic oxide suspensions in a salt containing medium were markedly different from those which were apparent in ion free water. Over 90% DNA binding was observed with both of the metallic oxides in the ion containing medium in contrast to 5.6 to 7.2% which was observed in the ion free medium (Table1). The DNA binding to the metallic oxide particles

that were added as dry powders was greater than 90%. The binding of RNA was over 90% for both the dry forms and the for the suspension forms of the oxides.

As shown in Table 3, very little binding (5.2-6.7%) of DNA to the metallic oxide surfaces was noted when suspensions of the respective oxides originally present in deionized water were added to solutions of DNA in a salt containing medium. However, under these conditions over 90% of the RNA was bound. The results are summarized as follows:

Fumed metallic oxides, when added as dry powders to either ion free water solutions of DNA or RNA, or to salt solutions of DNA or RNA will bind large amounts of both of these nucleic acids.

Fumed metallic oxides in suspension form in ion free water will not bind significant amounts of DNA when added to ion free aqueous solutions of DNA or to the DNA contained in a salt solution, but will bind large amounts of RNA when added to ion free aqueous solutions of RNA and to salt solutions containing RNA.

Fumed metallic oxides in suspension form in salt solutions will bind large amounts of DNA and RNA when added to either ion free aqueous solutions or to salt solutions containing these two nucleic acids.

As previously mentioned, fumed metallic oxide particles acquire a strong positive charge when dispersed in water. This is an energetically unfavorable situation since it results in severe electrostatic repulsions between particles accompanied by an increase in the energy of thermal fluctuation. It was noted that when a suspension of either fumed aluminum oxide or fumed titanium oxide in deionized water was added to a solution of DNA in deionized water (Table 1) or to a solution of DNA in sodium chloride, (Table 3) very little or no metallic oxide DNA complex was formed. Since DNA binds to the metallic oxide surface by a bridging mechanism,

this situation may be likened to "an attempt to construct a suspension bridge during a severe earthquake when the very foundations are rattling". In contrast, when RNA, a much smaller molecule was employed in place of DNA, significant binding of the RNA to the metallic oxide surface was evident. Most conspicuously, the dry fumed metallic oxide powders when added to either ion free water solutions or salt containing solutions of DNA and RNA show marked binding properties for these nucleic acids. On the basis of these observations, a tentative hypothesis is proposed to explain this behavior.

Immediately upon contact with the aqueous solution, a small number of water molecules become bound to the previously dry particle and an instantaneous permanent partial positive charge develops on the particle surface. The electrostatic repulsive force between particles at this stage of hydration is not too severe, and may be attenuated or neutralized by binding to the negatively charged nucleic acids. As more water molecules become bound, the number of positive charges progressively increases. When this occurs, more nucleic acids become bound to the surface and attenuate the developing charges. The end result is a nucleic acid-metallic oxide complex in high yield.

The marginal affinity of DNA and the pronounced affinity of RNA for fumed metallic oxides dispersed in ion free water provides a means for removing unwanted RNA from DNA in DNA purification procedures. Current DNA processing technologies employ RNA degrading enzymes which are expensive and ion sensitive. In contrast, the fumed metallic oxide suspensions in deionized water are cost effective and do not appear to be ion sensitive.

The ability of fumed metallic oxides to bind both DNA and RNA when added to solutions of these nucleic acids as a dry powder provides a good indication that this oxide could be employed to remove contaminating nucleic acids in the downstream processing of recombinant proteins. However, the

practical utility of dried powders in the fields of forensics, diagnostics, and recombinant DNA technologies are severely limited, since the procedure necessitates the weighing out of multiple portions of these powders which are to be added to many samples of DNA containing material; a procedure which is laborious, time consuming and very expensive. On the other hand, the use of suspensions of fumed metallic oxides dispersed in a salt solution is highly desirable, since they can be easily pipetted, are stable, economical, user friendly, and are amenable to automation.

Since charge repulsion between the metallic oxide particles suspended in ion free water prevents DNA from binding to them, it was found necessary to attenuate or to neutralize the positively charged surface (Figure 1) to an extent so as to permit DNA-metallic oxide bridge formation. It was found that when DNA binding experiments were conducted using metallic oxide suspensions dispersed in sodium chloride solutions, the positive charges were attenuated as evident by DNA binding.

Based upon current theories regarding electrified surfaces, it is postulated that the metallic oxide surface was altered by replacing some of the counterfield water molecules with anions (Figure 2). Anions differ from cations in their surface activity because they are usually not hydrated. Just as in the case of a water air interface where the anions are found preferentially at the surface, anions will move in toward the hydration sheath of the partially charged particle and displace a water molecule. Accordingly, a number of anions were tested for their ability to shield the charged surface.

Materials: Aluminum Oxide C, Titanium Oxide P-25

Inorganic salts: Aqueous solutions of the sodium salts of the following anions: Chloride, sulfite, sulfate, bicarbonate, tetraborate and hydrogen phosphate were prepared at an ionic strength of 0.15.

Organic salts: Aqueous solutions of the sodium salts of the following anions: Acetate, oxalate, and citrate were prepared at an ionic strength of 0.15.

DNA: Polymerized calf thymus DNA at concentrations of 50 micrograms per milliliter were prepared in the respective salt solutions.

Procedure:

Aluminum Oxide C (2.5 grams) and Titanium Oxide P25 (2.5 grams) were added to 100 milliliter volumes of the respective salt solutions to yield 2.5w/v % suspensions.

One and one half-milliliter volumes of the metallic oxide suspensions were added to one milliliter volumes of the respective nucleic acids. The tubes were mixed by inversion for about 10 seconds and then centrifuged at 10,000 x g for 5 minutes. The supernatants were recovered and analyzed for the presence or absence of DNA by UV absorption at 260 and 280 nanometers.

The extent to which the positive charge on the metallic oxide surface was attenuated by the added counter anion is reflected by the amount of free DNA present in the supernatant of this mixture. The presence of high DNA concentrations in the supernatant after its exposure to the oxide surface indicates that the positive charges on the oxide surface were effectively neutralized by the anion and were therefore unavailable for DNA binding. On the other hand, weak charge attenuation by the counter anion, which is the desired effect makes more sites available for DNA binding. In this latter case, less DNA will be present in the supernatant. The results of the experiment are summarized in Table 4.

Table 4

Attenuation of charges on metallic oxide surfaces by the anions of sodium salts.

Counter Anion	Amount of DNA Bound to Aluminum Oxide C (%)	Amount of DNA Bound to Titanium Oxide p-25 (%)
Chloride Cl^-	92.4	90.4
Sulfite SO_3^{2-}	88.2	86.4
Sulfate SO_4^{2-}	82.7	80.4
Bicarbonate HCO_3^-	80.2	80.0
Phosphate HPO_4^{2-}	21.2	18.4
Tetraborate $B_4O_7^{2-}$	38.7	38.2
Acetate CH_3COO^-	90.2	89.4
Oxalate $[OOC\text{-}COO]^{2-}$	82.3	75.2
Citrate $[OOCCH_2C(OH)COOCH_2COO]$	78.4	70.4

As shown in Table 4, the chloride and acetate ions appear to be the preferred anionic species for optimal charge attenuation since maximal DNA binding (92.4% to Al oxide, 90.4% to Ti oxide for chloride, and 90.2% to Al oxide, 90.0% to Ti oxide for acetate) to the surface occurs in their presence. There seems to be some marginal correlation between the number of units of negative charge present on the different organic carboxylate ions and ability to neutralize the oxide surface. The effectiveness increases in the order

citrate^{3-} > oxalate^{2-} > acetate^{1-} as evidenced by an increase in DNA binding with decreasing charge.

In the case of the inorganic anions, the valence of the species is not the only factor that is responsible for charge attenuation. Anions with the same number of unit charges exhibit strikingly different activities when exposed to the metallic oxide surface. For example, sulfate which has 2 units of negative charge is much less effective than hydrogen phosphate (HPO_4^{2-}) and tetraborate ($B_4O_7^{2-}$) in neutralizing the positive charge on the metallic oxide surface. This is evidenced by the fact that less DNA is bound in the presence of HPO_4^{2-} and $B_4O_7^{2-}$ than in the presence of SO_4^{2-}.

In order to explain the behavioral differences of the identically charged species, the electronegativities of the constituent atoms of the respective anions were taken into account. Electronegativity is the property of an atom describing its ability to attract an electron pair. Oxygen is the most electronegative and is assigned a value of 3.45, followed by Nitrogen (2.98), Carbon (2.55), Sulfur (2.53), Hydrogen (2.13) and Phosphorus (2.10) and Boron (2.0). Accordingly, the negative charge density surrounding the oxygen atoms involved in the O-P bond in the HPO_4^{2-} ion or the oxygen atoms involved in the $B_4O_7^{2-}$ ion would be greater than the negative charge density surrounding the oxygen atoms in the O-S bond in the SO_4^{2-} ion or any of the other counterions used in the present invention. Therefore, surface charge attenuation by anions is largely dependent on the chemical nature of the atoms that constitute the ionic species.

Dissociation of DNA-fumed metallic oxide - DNA complexes

An attempt was made to dissociate the metallic oxide-DNA complex in order to recover the free nucleic acid. Since the chloride ion was found to be a suitable charge attenuation anion, complex formation between DNA and the metallic oxide surface was conducted in aqueous solutions of sodium chloride.

The complex was then dissociated under appropriate mild alkali conditions.

Procedure:

Aluminum oxide C(2.5 grams), titanium oxide P25 (2.5 grams) and VP zirconium oxide (2.5 grams) were added to 100-milliliter volumes of sodium chloride, ionic strength 0.15, to yield 2.5w/v % suspensions. One and one half-milliliter volumes of the metallic oxide suspensions were added to one-milliliter volumes of DNA containing 50 micrograms of DNA per milliliter of sodium chloride solution. The tubes were then inverted several times, and the complex that was formed was allowed to settle under unit gravity. The supernatant was discarded, and the complex was washed twice with using two five-milliliter volumes of deionized water. The tubes were then centrifuged at 1000 x g for 10 minutes after which time a tight pellet of the DNA-metallic oxide complex became evident. The tubes were then inverted to remove the residual supernatant.

The pellets were then separately treated with 1.0 milliliter volumes of the following solutions: 0.02M tribasic sodium phosphate pH 9.2, 0.02M sodium tetra-borate pH 9.5 and 0.02M sodium hydroxide pH 12.0. The DNA recoveries are reported in Table 5.

Table 5

DNA recovery using dissociating conditions

Complex	DNA recovery 0.02M PO_4^{2-}	DNA recovery 0.02M $B_4O_7^{2-}$	DNA recovery 0.02M OH^{1-}
DNA-Aluminum Oxide-C	26.4%	18.8%	32.4%
DNA-Titanium Oxide P-25	88.6%	86.2%	93.2%
DNA-VP Zirconium Oxide	74.2%	68.7%	82.4%

As shown in Table 5, the recovery of DNA from Aluminum Oxide C particles was poor (18.8% to 32.4%) regardless of the dissociating solution. On the other hand, good displacement from the Zirconium Oxide particles (68.7% to 82.4%) and the Titanium Oxide particles (86.2% to 93.2%) was evident using these dissociating conditions. On the basis of these results, further DNA studies were conducted using suspensions of titanium oxide P 25 and dissociating the complex with a 0.02 molar solution of sodium hydroxide.

Dispersed metallic oxides would appear to be suitable in situations where the use of traditional methodologies may prove to be cumbersome, expensive and even prohibitive.

Urine Screening

In a number of malignancies, most notably bladder cancer, abnormal cells are excreted in the urine. In order to determine whether a specific mutation had occurred, a large number of cells are collected, and the DNA is isolated by a lengthy expensive procedure. The DNA is then subjected to genetic testing using a probe that has the same mutation as the affected gene. A simple alternative method would employ dispersed metallic oxides to capture the DNA after its release from the cells in the presence of a chaotropic ion, followed by the dissociation of the metallic oxide - DNA complex in order to recover the intact DNA.

Procedure

The urine sample is collected and guanidine thiocyanate added to the sample to a final concentration of 3M. Guanidine thiocyanate is a chaotropic agent, and functions by disrupting the cell and the nucleohistone complex, and thereby releasing free DNA into the aqueous media. The solution is then diluted with water to a final concentration of 1.5M with respect to guanidine thiocyanate.

A suspension of metallic oxide in 0.15M sodium chloride is then added to form an aggregate of metallic oxide and DNA. The aggregate is then allowed to settle to the bottom of the tube. Alternatively the tube can be subjected to low speed centrifugation and the aggregate recovered as a tight pellet.

The DNA-metallic oxide complex is washed three times with deionized water after which it is dissociated with 0.02M solution of sodium hydroxide and neutralized with a 0.1M solution of Tris HCl.

There are many diagnostic kits on the market that are used for the isolation of DNA from whole blood, however, a majority of them employ organic solvents (e.g. phenol, chloroform, isopropanol) that requires a lot of "hands on time" and are expensive.

Utilizing the principles of the present invention the following protocol is employed for this purpose. In this procedure, one volume of whole blood is treated with two volumes of a chaotropic agent such as 3M guanidine thiocyanate in a buffer, say, 100 mM sodium acetate pH 7.0. After standing at room temperature for 15 minutes a suspension of the protein precipitator ProCipitate™ (manufactured by Ligochem Inc., Fairfield NJ) is then added to precipitate the protein. The composition of ProCipitate is disclosed in U.S. Patent Nos. 5,294,681; 5,453,493; and 5,534,597, and U.S. Application Serial No. 08/676,668 (now allowed) incorporated herein by reference in their entireties.

The tubes are then centrifuged at 10,000 x g for 15 minutes, and the supernatant recovered, 1.5 volumes of Titanium Oxide P-25 is then added. The resulting aggregate consisting of DNA and metallic oxide is allowed to settle under unit gravity. After settling the supernatant is removed by aspiration and the settled complex is washed with three washings using deionized water. The tubes are then centrifuged at 1000 x g for 30 seconds. The supernatant is discarded and 0.02M sodium hydroxide is added to the tube.

The tubes are then vortexed, followed by centrifugation at, say, 10,000 x g for 5 minutes. The supernatants are then removed neutralized with a 0.1M Tris HCl solution and analyzed for DNA by spectrophotometric absorption at 260 and 280 nm. One ml of whole blood contains approximately 40 to 50 micrograms of DNA. This quantity translates into about one absorbance unit (AU) at 260 nm and 0.8 AU at 280 nm. The DNA specimens are also subjected to agarose gel electrophoresis in which the DNA bands were identified by ethidium bromide staining.

In another version of this procedure one volume of blood is treated with two volumes of 3M guanidine thiocyanate in 100 mM sodium acetate (EDTA is not present). The mixture is then heated at 65 degrees Celsius for 10 minutes. After standing at room temperature for 5 minutes, a suspension of ProCipitate™ is added to precipitate the protein. The supernatant is recovered by centrifugation and this DNA containing solution is processed and analyzed for DNA as described above.

Alternatively, one volume of whole blood is treated with three volumes of a 1.0% w/v of sodium dodecyl sulfate (SDS) in a buffer, say, 10 mM solution of Tris buffer and 100mM EDTA pH 8.0. After remaining at room temperature for 15 minutes, 3 volumes of a 3M solution of potassium acetate is added to neutralize the SDS and to precipitate the hemoglobin that is present. The tubes are then centrifuged and the supernatant is recovered. 1.5ml of Titanium Oxide P-25 suspension is then added. The aggregate is allowed to settle under unit gravity. After settling the supernatant is discarded and the DNA-metallic oxide complex is washed with three washings of deionized water. The tubes were then centrifuged at 1000 x g for 30 seconds and the supernatant discarded. Dissociation of the complex was accomplished by the same method that was used in the ProCipitate™ guanidine thiocyanate procedure.

General procedure for the preparation of fumed Titanium Oxide P-25 Suspensions for the isolation of nucleic acids from biological fluids

It was determined that treatment of Titanium Oxide suspensions with polyalkylene glycols such as polyethylene glycol (PEG) or polypropylene glycol (PPG) increases the particle density, and thus permits the ensuing DNA-metallic oxide complex to settle more rapidly under unit gravity and to become more compact after settling than non PEG treated particles. More importantly, the PEG treated oxide which binds DNA to the same extent as the non-treated version, does not bind detectable levels of protein. Therefore, the PEG treated oxide is particularly suited for the isolation of DNA from proteinaceous biological fluids. In contrast, the untreated oxide settles more slowly in a protein containing medium, and was also found to occlude small quantities of certain proteins such as immunoglobulins. The molecular weight range of the polyalkylene glycols is 200-9000 daltons used in an amount of a 1-10% w/v aqueous solution.

Materials and Methods

30g Titanium Oxide P25 were dispersed in 300ml of 0.1M sodium hydroxide. The suspension was gently stirred for 30 minutes and then allowed to settle under unit gravity. The supernatant was removed by aspiration and discarded. The settled oxide was then treated with 300ml of 0.15M sodium chloride and mixed for 5 minutes. After settling, the supernatant was removed. The process of sodium chloride addition, mixing, followed by settling under unit gravity was repeated two more times. To the oxide was added 300ml of a 10w/v% solution of polyethylene glycol 8000 (Mw 7000-8000 daltons) in 0.9% sodium chloride. After thorough dispersion followed by mixing for 30 minutes the suspension was allowed to settle and the supernatant was discarded. The sediment was then washed with two 300ml volumes of 0.9% NaCl to insure complete removal of weakly bound polyethylene glycol.

Following this procedure, the oxide was dispersed in 250ml of 0.9% sodium chloride, the pH was adjusted to 7 by the slow addition of 0.1N HCL and the volume was adjusted to 300ml by the addition of 0.9% sodium chloride to yield a 3.0 w/v% suspension.

DNA isolation from biological fluids

Materials required

A solution of 3M guanidine thiocyanate, 100mM ethylene diamine tetra acetate, sodium salt (EDTA) and 100mM sodium acetate pH 7.0.

A 3M solution of guanidine thiocyanate in 100mM sodium acetate pH 7.0.

A 5M solution of guanidine thiocyanate 100mM EDTA in 100mM sodium acetate pH 7.0.

ProCipitate™

A suspension of 3.0 w/v% Titanium Oxide P25 in 0.9% sodium chloride and previously treated with a 10% aqueous solution of polyethylene glycol 8000 PEG. The surface area of the Titanium Oxide P25 particle ranges from 60-70 m/g, the average particle diameter is 30 nanometers.

Dissociation Buffer: A solution of 0.02M sodium hydroxide in deionized water.

Neutralizing Buffer: A solution of 0.1M Tris (hydroxymethyl) amino methane hydrochloride (Tris HCl) pH 4.8 in deionized water.

Alternate Procedure

A solution of 1.0 w/v% of sodium dodecyl sulfate (SDS) in a 10mM solution of Tris buffer and 100 mM EDTA pH 8.0.

(A solution of 3M potassium acetate in deionized water.

(A 0.1M solution of Tris HCl pH 4.8 in deionized water.

Example 1

DNA isolation from white blood cells in urine

Since urine from cancer bearing patients was not available, an example was performed using normal urine spiked with whole white blood cells and carrying out the procedure as below:

Isolation of white blood cells

One ml of whole mouse blood was added to 3ml or red cell lysing solution. The lysing solution consisted of 1 part of a solution containing 0.9% sodium chloride (saline) and 2 parts water. The tubes were mixed by repeated inversion for 1.0 minute, and then centrifuged at 2000 x g for 10 minutes. The supernatant which consisted mainly of lysed red blood cells and other serum components was removed by aspiration and discarded. The pellet which consisted of white blood cells and hemoglobin dispersed in 1.0ml of saline and recentrifuged in order to remove contaminating hemoglobin, and then resuspend in 1.0 ml of saline.

Lysing of white blood cells in the urine specimen to release free DNA

As previously stated DNA does not generally occur in a free form, but is bound instead to the histone fraction in nucleated cells. High concentrations of guanidine thiocyanate have the property of disrupting the nucleohistone complex with the concomitant release of DNA into solution.

One ml of white blood cell suspension were added to 10ml aliquots of normal urine. In one series of tubes, a 5M solution of guanidine thiocyanate in EDTA was added to a final concentration of 3M. After the dissociation of the nucleohistone complex had occurred to release free DNA, the urine specimen was diluted with water to achieve a final concentration of 1.5M (a non dissociating condition) with respect to guanidine thiocyanate. A second series of urine-

white blood cell containing tubes were not treated with the chaotrope, and were introduced for comparative purposes.

One ml aliquots of a 3.0% suspension of Titanium Oxide P25 (previously treated with polyethylene glycol 8000, PEG) were then added to the chaotrope treated and to the non-chaotrope treated urine sample. Immediately, upon addition white aggregates consisting of DNA and metallic oxide appeared in the treated specimen (Fig. 3). No aggregate formation was evident in the non-chaotrope treated preparation. After settling, the supernatant was removed by aspiration and 5.0ml of deionized water was added to the aggregate in order to remove contaminating protein and residual guanidine thiocyanate. The contents were mixed by 3 gentle inversions after which the supernatant was discarded. The aggregate was then washed further with 2x10 milliliter portions of distilled water with repeated inversion within a period of 5 minutes. After the final wash, the tube was centrifuged at 2000 x g for 5 minutes, and the residual liquid was removed by aspiration.

One ml of sodium hydroxide solution (0.02M pH12.0) was added to the pellet, and followed by repeated mixing with a micropipette in order to insure complete dissociation of DNA from the metallic oxide surface. The preparation was then centrifuged at 10,000 x g for 6 minutes. DNA was then recovered in the supernatant. The alkaline supernatant was acidified to pH 8.0 by the addition of 150 microliters of 0.1N Tris HCl.

The absorbance at 260nanometers was 0.90 and the absorbance at 280 nanometers was 0.52. These values translate into a 260:280 ratio of 1.73 with a yield of about 90%.

After electrophoresis in agarose gels, a single sharp fluorescent band was observed with a molecular mass greater than 20 kilobases (K_6). The DNA was also subjected to repeated amplification cycles using the Polymerase Chain Reaction (PCR) and was found to have the same properties as highly purified DNA obtained.

Procedure

To 100 microliters of whole mouse blood was added 300 microliters of red cell lysing solution (consisting of one part 0.9% saline and 2 parts of distilled water). The tubes were mixed by inversion and allowed to stand at room temperature for 5 minutes. After centrifugation at 2000 x g, the supernatant was discarded and the pellet was recovered. Guanidine thiocyanate-EDTA solution (100 microliters) was added to the pellet. The contents were then thoroughly mixed with a micropipette using repeated up and down motion and allowed to stand at room temperature for 5 minutes.

ProCipitate™ (200 microliters) was then added to the dispersed pellet followed by vortexing for 10 seconds. The tube was then centrifuged at 10,000 x g for 5 minutes after which time the supernatant was recovered. Titanium Oxide suspension (150 microliters) was then added to the DNA containing supernatant. The resulting aggregate consisting of DNA and titanium oxide was allowed to settle under unit gravity. After settling, the supernatant was removed by aspiration and 1.0 milliliter of deionized water was added to the aggregate in order to remove contaminating protein and residual guanidine thiocyanate . The contents were mixed by gentle inversion, after which the supernatant was discarded. The aggregate was then washed further with 2 x 10 milliliter portions of deionized water by repeated inversion. After the final wash, the tube was centrifuged at 3000 x g for 5 minutes and the residual liquid was removed by aspiration. Eighty microliters of 20 millimolar (mM) sodium hydroxide was added to the pellet, and followed by repeated mixing with a micropipette in order to insure complete dissociation of DNA from the metallic oxide surface. The preparation was then centrifuged at 10,000 x g for 6 minutes. DNA was then recovered in the supernatant. The alkaline supernatant was acidified to pH 8.0 by the addition of 10 microliters of 0.1M Tris HCl.

The absorbance at 260 nm was 0.76 and the absorbance at 280 nano-meters was 0.43. These values translate into a 260:280 ratio of 1.77 with a yield of 76%.

Alternate Procedure

To 100 microliters of whole mouse blood was added 300 microliters of red cell lysing solution, the tubes were mixed by inversion and allowed to stand at room temperature for 5 minutes. After centrifugation at 2000 x g for 10 minutes the supernatant was discarded and the pellet was recovered. Sodium dodecyl sulfate (SDS) solution (100 microliters) was added to the pellet. The contents were then thoroughly mixed with a micropipette using repeated up and down motion and allowed to remain at room temperature for 5 minutes.

Potassium acetate (100 microliters) was then added to the solubilized pellet, followed by vortexing for 10 seconds. The tube was then centrifuged at 10,000 x g for 5 minutes after which the supernatant was recovered. Titanium Oxide suspension (150 microliters) was then added to the DNA containing supernatant. After settling and removal of the supernatant, the aggregate was washed with three 500 microliter portions of deionized water, followed by removal of the supernatant after each wash. Further processing was achieved as described for the guanidine thiocyanate-ProCipitate™ procedure.

Analysis of the final product by UV absorption at 260 and 280 nanometers revealed a ratio of 1:6 indication that the product was not as pure as the product obtained by the guanidine thiocyanate-ProCipitate™ method. Nonetheless, repeated amplification by PCR generated a product that was comparable to pure genomic DNA.

Example 3

Procedure for the removal of contaminating RNA from DNA

The weak affinity of fumed metallic oxide particles

(previously dispersed in deionized water) for DNA and the strong affinity for RNA makes this reagent a suitable candidate for removing unwanted RNA in DNA purification protocols.

As an example, the biological sample is first extracted with a suitable reagent that will release both DNA and RNA into the aqueous medium. Fumed metallic oxide particles, that have been previously dispersed in deionized water are added to the treated sample to adsorb RNA. The mixture is then centrifuged, and the supernatant containing the DNA is recovered. Fumed metallic oxide particles that have been previously dispersed in a salt solution are then added to capture DNA. The DNA-metallic oxide complex is then washed, and dissociated to release free DNA, followed by neutralization.

The Capture and Quantification of DNA in Urine Specimens Containing Appreciable Quantities of Blood Proteins

Since bleeding occurs in many patients with malignancies (e.g. bladder cancer, and in patients who have succumbed to other pathologies), the voided urine may contain copious amounts of hemoglobin and other proteins which could interfere with DNA analysis. This problem was addressed and it was found that purified DNA can be isolated from problem urine and subsequently quantified by using the procedure disclosed below.

Preparation of urine sample

One milliliter of whole mouse blood was treated with 3 ml of red cell lysing solution. The lysing solution consisted of 1 part of a solution containing 0.9% sodium chloride and 2 parts water. The tubes were mixed by inversion, allowed to stand at room temperature for 10 minutes, and then centrifuged at 2000 x g for 10 minutes. The supernatant, which consisted

mainly of lysed red blood cells and other serum components, was removed by aspiration and discarded. The pellet consisted of white blood cells and large amounts of residual blood components. The quantity of hemoglobin in the pelleted fraction, as estimated spectrophotometrically, was found to be 7.0 milligrams. The urine specimen was prepared by adding 0.3 grams of lyophilized male urine (Sigma) to 9.0 ml of distilled water. This solution was then added to the white blood cell pellet and inverted several times to insure proper mixing.

Dissociation of the nucleohistone complex to release free DNA

DNA does not generally exist in a free form, but is bound instead to the histone fraction in nucleated cells. Dissociation of the nucleic acid-histone complex is achieved by treatment with a chaotropic reagent. This treatment results in cell disruption and dissociation of the DNA histone complex to release free DNA into the solution.

Twelve milliliters of a 5M solution of guanidine thiocyanate (GuSCN) in 0.01M sodium acetate pH 7.0 containing 100mM EDTA was added to the urine sample (9.0ml) and the tube was mixed by repeated inversions for 15 minutes.

Precipitation of extraneous proteins

Contaminating proteins were removed by treating the chaotrope containing urine with a water insoluble cross-linked polymeric acid, trade name ProCipitate™. This product is described in US Patents Nos. 5,294,681/5,453,493 and 5,534,597, and is supplied as a 2.8-3.1 w/v% suspension in water.

Five milliliters of ProCipitate™ were added to the chaotrope treated urine. The tube was inverted several times and then allowed to remain at room temperature for five minutes. The tube was then centrifuged at 1500 x g for 10 minutes and the supernatant recovered.

DNA capture and DNA Desorption

One and one half milliliters of a 3.0 w/v% suspension of Titanium Oxide P25 was added to the treated urine. Immediately, upon addition, white aggregates consisting of DNA and DNAble appeared. After settling under unit gravity for 5 minutes, the supernatant was removed and discarded. Five milliliters of deionized water was added to the pellet. The tubes were inverted several times and the supernatant discarded. The process of washing the contents with water was repeated two more times. After the final wash, the tube was centrifuged at 3000 x g for 10 minutes. The tube was then inverted to allow for removal of excess liquid.

Eight hundred microliters of a 20 millimolar (mM) solution of sodium hydroxide was then added. The contents of the tube were mixed using the up and down motion of a micropipette. The contents were then transferred to four microcentrifuge tubes equipped with a 0.2 micrometer cellulose acetate membrane filter and centrifuged at 2500 x g for 10 minutes. The filtrates were pooled, followed by the addition of 200 microliters of 0.1M Tris HCl.

The absorbance at 260 nanometers was 0.85 and the absorbance at 280 nanometers was 0.46. This translates into a 260:280 ratio of 1.85 with a yield of about 85%.

After electrophoresis in agarose gels, a single sharp fluorescent band was observed with a molecular mass greater than 20 kilobabses (kb). The DNA was also subjected to repeated amplification cycles using the Polymerase Chain Reaction (PCR) and was found to exhibit a similar electrophoretic profile as highly purified DNA.

The Capture and Quantification of DNA from Whole Blood

To 50 microliters of whole mouse blood was added 100 microliters of a 3M solution of guanidine thiocyanate in 0.1M sodium acetate and 100mM EDTA pH 7.0. The solution was vortexed and then incubated at room temperature for ten minutes. Two hundred and fifty microliters of ProCipitate™

suspension was then added. The contents were then vortexed for 5 seconds and then mixed further by repeated inversions for 5 minutes. After centrifugation at 10,000 x g for 5 minutes the supernatant was withdrawn and placed into a clean 1.0ml centrifuge tube. Seventy-five microliters of Titanium Oxide P25 suspension was then added. The tube was gently inverted, and the aggregate consisting of nucleic acid complexed to the oxide was allowed to settle under gravity. The supernatant was carefully aspirated and discarded. The pellet was washed twice with 500 microliter portions of deionized water. After the final wash, the pellet was centrifuged at 3000 x g for 5 minutes, and the residual liquid was removed. The DNA was dissociated from the complex by thoroughly dispersing the pellet in 40 microliters of 20 millimolar (mM) sodium hydroxide using a micropipet. The suspension was then centrifuged through a 0.2 micrometer membrane filter at 3000 x g for 10 minutes. The filtrate containing DNA was then neutralized with 5 microliters of 0.1M Tris HCl.

The absorbance at 260 nm was 0.65 and the absorbance at 280 nano-meters was 0.36. These values translate into a 260:280 ratio of 1.8 with a yield of 65%. After electrophoresis in agarose gels, a single sharp fluorescent band was observed with a molecular mass greater than 20 kilobases (kb). The DNA was also subjected to repeated amplification cycles using the Polymerase Chain Reaction (PCR) and was found to have the same properties as highly purified DNA.

The isolation of DNA from whole blood may be performed by heating the sample in the presence of guanidine thiocyanate in the absence of the metal chelator EDTA. The procedure was performed as follows: To 50 microliters of whole mouse blood was added 100 microliters of a 3M solution of guanidine thiocyanate in 0.1M sodium acetate pH 7.0. The solution was then vortexed and incubated at 65°C for 10 minutes. After

cooling to room temperature, 250 microliters of ProCipitate™ was added. The sample was then processed using the same procedure that was employed for the isolation of DNA in the presence of EDTA using room temperature conditions and which is described above.

The absorbance at 260 nm was 0.70 and the absorbance at 280 nanometers was 0.38. These values translate into a 260:280 ratio of 1.8 with a yield of 70%. After electrophoresis in agarose gels, a single band was observed with a molecular mass greater than 20 kilobases (kb). The DNA was found to be suitable for amplification by Polymerase Chain Reaction (PCR). Agarose Gel Electrophoresis

After the DNA was isolated, its presence was verified by electrophoresis in 0.75% agarose gel followed by staining with ethidium bromide. Ten microliters of the isolated DNA was added to 3 microliters of DNA loading dye (bromophenol blue-xylene cyanol dye, 25.0% Sigma in 50% glycerol). Ten microliters of this mixture was loaded onto the gel. The gel was run for 45 minutes at 75 volts after which time the DNA band was detected using short wave ultraviolet light. (Figure 4).

Polymerase Chain Reaction

Eight microliters of the isolated DNA was amplified using the Taq PCR core kit purchased from Qiagen (Valencia, CA). Mouse Histone H^3 primer was supplied by Dr. R. Donnelly, University for Medical and Dentistry, Newark, New jersey; the size of the amplicon (bp) that was generated is 370 base pairs (bp). Along with the test sample, a positive control of 100 nanograms of purified mouse DNA was subjected to amplification.

The reaction was carried out according to the instructions in the PCR handbook from Qiagen (Taq PCR Handbook, Page 10, May 1997). The following cycling program was used:

Initial Denaturation 3 step cycle	3 min	94°C
Denaturation	45 sec	94°C
Annealing	45 sec	58°C
Extension	1 min	72°C
Cycles	25	
Final Extension	7 min	72°C

The product after amplification was subjected to agarose gel electrophoresis using the conditions described above. The electrophoretic profile is shown in Figure 5.

PCR amplification of small quantities of DNA present in plasma

An attempt was made to capture and release minute amounts (less than $1x10^3$) of DNA from a highly proteinaceous medium such as plasma using the fumed metallic oxide process.

Procedure:

Two hundred and fifty microliters of filtered plasma pH 7.2 was mixed with 250ul of 2.5M guanidine thiocyanate in 50mM sodium acetate pH 7.0. Two microliters of human genomic DNA (58 nanograms or $1.6x10^3$ copies) was added to the above solution. After gentle mixing 75ul of the Titanium Oxide P-25 suspension was added. The tube was then mixed and centrifuged at 5,000 x g for 5 minutes. The supernatant was then discarded. The pellet was then dispersed in 400ul of diethylpyrocarbonate (DEPC) treated water (1 part DEPC: 1000 parts of deionized water), centrifuged at 5,000 x g for 5 minutes. The supernatant was discarded. The pellet was washed two more times with DEPC treated water. The pellet was then treated with 40 microliters of a 0.02M solution of sodium hydroxide followed by mixing with a micropipet using up and down motion to dissociate the complex. The contents was transferred to a microfilter (0.22um) centrifuge tube and spun

at 3,000 x g for 10 minutes. To the filtrate was added 7ul of 0.1N Tris HCl pH 4.8. Two microliters of this preparation was amplified by the Polymerase Chain Reaction (PCR) using the reagents and amplification protocol of Qiagen (Taq PCR Hand Book, Page 12, May 1997). Human ß actin primer was purchased from Perkin Elmer Catalog #: N808-0230, Foster-City, California; the size of the amplicon generated is 294 bp. The following cycle program was used:

Initial Denaturation	3 min	94°C
3 step cycle		
Denaturation	45 sec	94°C
Annealing	45 sec	58°C
Extension	1 min	72°C
Cycles	30	
Final Extension	10 min	72°C

The product after amplification was subjected to agarose gel electrophoresis using the conditions described above. The electrophoretic profile is shown in Figure 6.

Isolation of BAC clones for sequencing

DNA libraries constructed in bacterial artificial chromosome (BAC) vectors propagated in E. coli bacteria have become the choice for clone sets in high through put genomic sequencing projects, primarily of their high stability. BAC is an acronym. BAC stands for Bacterial Artificial Chromosome. These are large circular constructs of double stranded DNA similar to plasmids but many times larger. BACs are on the order of 120 to 300 kb in diameter as compared with 2kb for regular plasmids.

The institute for Genomic Research Rockville, MD has developed a 96 well template purification procedure using alkaline lysis and the protein precipitating agent

(ProCipitate™) to obtain purified BAC preparations in high yield. This method is still time consuming and cumbersome since it requires alcohol precipitation and centrifugation in a specialized microtiter plate holder. An attempt was therefore made to simplify the method by replacing the alcohol treatment step by one that employs saline suspensions of Titanium Oxide P-25.

Procedure

E. coli containing the BAC construct were grown in Luria Broth (LB) as described by Kelley J.M. et al. (Nucleic Acids Research, 27, 1539-1546, 1999).

Thirty milliliters of the BAC culture were centrifuged at 1500 x g for 10 minutes. The supernatants were decanted and the bacterial pellet was dispersed in 1.25 milliliters of 10mM Tris buffer containing 5.0mM EDTA. Seventy-five microliter aliquots of the bacteria suspension were subsequently employed in all studies. Seventy-five microliters of lysis solution (1.0% sodium dodecyl sulfate in 0.2N sodium hydroxide) was added to each tube. The tubes were mixed by inversion and allowed to stand at room temperature for about 5 minutes. Seventy-five microliters of a 3M solution of potassium acetate pH5.8 was then added. The tubes were mixed followed by the addition of 75ul of ProCipitate™. The tubes were vortexed , allowed to stand at room temperature and then centrifuged at 14,000 x g for 5 minutes. The supernatants were carefully removed and treated with 6 microliters of a 0.01% solution of RNAse in water in order to degrade the RNA present. Seventy-five microliters of an aqueous suspension (3.0%) of Titanium Oxide P-25 was then added. After settling under unit gravity, the BAC DNA-metallic oxide was washed with distilled water. Further processing and isolation of BAC DNA was achieved by the procedure employed for blood and urine.

The electrophoretic profiles of the BAC DNA isolated by the fumed metallic oxide process and the alcohol-

centrifugation procedure were compared (Figure 7).

Test kits involving the method of the present invention

The methods of the present invention can be conveniently practiced prepared test kits. In a preferred embodiment a kit for use in a method for isolating deoxyribonucleic acid (DNA) from other substances in solution, includes, comprises, consists of or consists essentially of (A) a substrate for holding a specimen, (B) a chaotrope, (C) a protein precipitating agent, (D) an adsorbent comprising a fumed metallic oxide, and (E) a DNA dissociating agent an example of a chaotrope is guanidine thicyanate. The chaotrope may include guanidine thicyanate and a buffer. As indicated herein, the protein precipitating agent is preferably Procipitate™. The DNA dissociating agent is preferably a 0.02 molar solution of sodium hydroxide. The adsorbent is preferably a fumed metallic oxide selected from the group consisting of aluminum oxide, titanium oxide, and zirconium oxide. The fumed metallic oxide may be used in combination with an aqueous solution of sodium salts of an anion selected from the group consisting of acetate, bicarbonate, sulfate, sulfite, chloride, oxalate, and citrate. The fumed metallic oxide is preferably used in combination with 0.15M NaCl. The fumed metallic oxide may be used in combination with deionized water.

In the kit of the invention, the dissociating agent is preferably selected from the group consisting of 0.02M PO_4^{2-}, 0.02M B_4O^{2-} and OH^{1-}. The dissociating agent may alternatively be sodium hydroxide. The kit may further comprise a neutralizing buffer. The neutralizing buffer may be TrisHCl in deionized water.

As indicated, the adsorbent may be fumed metallic oxide. In an alternative embodiment the adsorbent includes a fumed metallic oxide and is attached to a solid-phase carrier. The solid-phase carrier can be a plastic bead or a microtiter

plate. The solid-phase carrier may be composed of insoluble polymers. The solid-phase carrier may be a polymer of styrene or vinyl chloride or a copolymer thereof. The solid phase carrier may take any form, such as a container, a stick or bead.

Example

General screening of blood samples in a 96 well-automated microtiter plate format for genetic aberrations; Isolation of Genomic DNA Experimental Protocol based on Genomic 96 well filter format (See Figures 8 and 9).

To 50 microliters of whole mouse blood, add 100 microliters of a 3M solution of guanidine thiocyanate in 0.10M sodium acetate, tape seal the plate and vortex for 30 seconds, incubate 10 minutes at 65°C and vortex again briefly. Shake ProCipitate well to completely resuspend. Using a wide bore pipette tip, add 250 uls of ProCipitate™ suspension to each well, mix by pipetting up and down 8-10 times to insure that each sample is homogeneous. Note: Failure to mix thoroughly may result in improper filtration.

Incubate for 5 minutes at room temperature.

Transfer each sample to the corresponding well of the Polyfiltronics 800 (Membrane GF/D) filter plate and vacuum filter. The collected supernatant contains the nucleic acids. Add 10ul of the RNase containing solution to each well and incubate at room temperature for 5 minutes

Transfer the supernatant to a 0.22um Millipore (Membrane MAGV N22) filter plate and add 75 microliters of *DNAble suspension (a water insoluble nucleic acid capture reagent) to each well containing the supernatant, mix well by pipetting up and down 8-10 times.

Apply vacuum again and filter, discard the supernatant.

Wash the aggregate consisting of nucleic acid complexed to *DNAble by the addition of 2 x 200ul volumes of distilled water to each well followed by vacuum filtration after each

addition.

Prepare a fresh solution of 20 millimolar sodium hydroxide, add 40 ul of the 20 millimolar solution of sodium hydroxide to each well and mix by up and down motion 8-10 times.

Finally, obtain a new receiver plate and vacuum filter; the final supernatant contains the nucleic acids.

Neutralize the solution in each well with 5 ul of 0.1M Tris HCL pH 4.8.

The kits and process of the present invention can advantageously be used in forensic medicine, molecular bioinformatics, in an automated systems for the isolation of bacterial and viral constructs for genomic sequencing, for non-invasive diagnostics, capture and quantification of DNA in saliva-capture, quantification of small quantities of DNA present in large volumes of urine, and removal of contaminating nucleic acids in the downstream processing of recombinant proteins.

Throughout the present specification where compositions, kits, and methods are described as including or comprising specific components, it is contemplated by the inventors that compositions of the present invention also consist essentially of, or consist of the recited components.

The purpose of the above description and examples is to illustrate some embodiments of the present invention without implying any limitation. It will be apparent to those of skill in the art that various modifications and variations may be made to the composition and method of the present invention without departing from the spirit or scope of the invention. All patents and publications cited herein are incorporated by reference in their entireties.

Claims

1. A method for isolating deoxyribonucleic acid (DNA) from other substances in solution using fumed metallic oxides, comprising:

(A) combining the fumed metallic oxides with the DNA containing solution to form a DNA-metallic oxide complex,

(B) allowing the DNA-metallic oxide complex to settle under unit gravity or by centrifugation,

(C) washing the DNA-metallic oxide complex with deionized water,

(D) dissociating the insoluble DNA-metallic oxide complex with mild alkali to release free DNA and forming a DNA containing solution,

(E) recovering the free DNA by centrifugation or by filtration, and

(F) neutralizing the DNA containing solution with either an acid or acid salt.

2. The method of claim 1, wherein the fumed metallic oxide is in the form of a dry powder or a suspension in a salt solution.

3. The method of claim 1, wherein the fumed metallic oxide is in the form of a suspension in a salt solution.

4. The method of claim 1, wherein the fumed metallic oxide is in the form of a dry powder.

5. The method of claim 1 wherein the DNA is in a form selected from the group consisting of, in a free form in solution; complexed to a protein, a lipid, or a carbohydrate; and a lysate of bacteria, virus, animal tissue, vegetable tissue or cells.

6. A method for isolating DNA from a proteinaceous medium, comprising

(A) treating the specimen with a chaotropic agent containing a metal chelator, or alternatively heating the specimen in the presence of the chaotropic agent without the chelator being present,

(B) adding a water insoluble protein aggregating agent, ProCipitate™ and isolating a liquid phase,

(C) treating the liquid phase with an adsorbent consisting of alumina, titania or zirconia generated by flame hydrolysis,

(D) separating the supernatant,

(E) washing the residue with deionized water,

(F) removing deionized water wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate or phosphate or a metal hydroxide, and

(G) recovering and neutralizing the liquid phase containing DNA.

7. The method of claim 6, wherein the proteinaceous medium is selected from the group consisting of whole blood, hemoglobin containing urine and saliva.

8. The method of claim 6, wherein the adsorbent consisting of alumina, titania or zirconia generated by flame hydrolysis is a dry powder.

9. The method of claim 6, wherein the adsorbent consisting of alumina, titania or zirconia generated by flame hydrolysis is a suspension of oxide in a salt solution which has weak charge attenuating properties, and where the salt solution is inorganic a suitable anion is chloride, and where the salt solution is organic, acetate is used.

10. A method for isolating DNA from whole blood comprising

(A) lysing the specimen and recovering a nucleated cell fraction,

(B) treating the nucleated cell fraction with a chaotropic agent containing a chelator, or treated with a chaotrope and heated without the chelator being present,

(C) treating the product of step (b) with either fumed alumina, titania, or zirconia in a dry powder form, or as a suspension in a salt solution which has weak charge attenuating properties,

(D) separating the supernatant,

(E) washing the residue with deionized water,

(F) removing the wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate or phosphate or a metal hydroxide, and

(G) recovering and neutralizing the liquid phase containing DNA.

11. A method for isolating DNA from whole blood, comprising

(A) lysing the blood sample and recovering a nucleated cell fraction,

(B) treating the nucleated cell fraction with a surfactant,

(C) adding potassium acetate to neutralize the surfactant and isolating a liquid phase,

(D) treating the liquid phase with alumina, titania, or zirconia as a dry powder or a suspension in a solution of a metal salt which has weak charge attenuating properties,

(E) separating the supernatant,

(F) washing the residue with deionized water,

(G) removing deionized water wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate or phosphate or a metal

hydroxide, and

(H) recovering and neutralizing the liquid phase containing DNA.

12. The method of claim 11, wherein the surfactant is 1.0% aqueous sodium dodecyl sulfate (SDS) containing metal chelator EDTA.

13. A method of isolating DNA from cells contained in a proteinaceous medium or from a medium which has substantially no protein, comprising

(A) treating the proteinaceous medium or medium which has substantially no protein with a chaotropic agent containing a metal chelator, or alternatively, heating the specimen in the chaotropic agent in the absence of a chelator,

(B) treating a liquid phase product of step (a) with either dry powdered fumed alumina, titania, or zirconia, or a suspension of these oxides in a solution of a salt with weak charge attenuating properties,

(C) separating the supernatant,

(D) washing the residue with deionized water,

(E) removing the wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate or phosphate or a metal hydroxide, and

(F) recovering and neutralizing the liquid phase containing DNA.

14. The method of claim 13, wherein the proteinaceous medium is serum or plasma.

15. The method of claim 13, wherein the medium which has substantially no protein is urine.

16. A method for isolating DNA from specimen cells contained in a proteinaceous medium or from a medium which has

substantially no protein, comprising

(A) treating the specimen with a surfactant sodium containing a metal chelator.

(B) adding potassium acetate to neutralize the surfactant and isolating a liquid phase,

(C) treating the liquid phase with dry fumed alumina, titania, or zirconia, or with metallic oxides in suspension in a solution of a metal salt with weak charge attenuating properties,

(D) separating the supernatant,

(E) washing the residue with deionized water,

(F) removing deionized water wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate or phosphate or a metal hydroxide, and

(G) recovering and neutralizing the liquid phase containing DNA.

17. A method for isolating free DNA from a specimen proteinaceous medium, comprising

(A) treating the specimen with dry fumed metallic oxide powders, or a suspension of the oxides in a solution of salt with weak charge attenuating properties, and

(B) processing the DNA metallic oxide complex to release DNA by separating the supernatant,

(C)) washing the residue with deionized water,

(D) removing deionized water wash and then dissociating the DNA from the fumed alumina, titania, or zirconia by treatment with aqueous alkali borate or phosphate or a metal hydroxide, and

(E) recovering and neutralizing the liquid phase containing DNA.

18. A method for isolating plasmids from bacterial lysates, comprising

(A) lysing the bacteria with sodium dodecylsulfate (SDS) in alkaline solution containing a metal chelator,

(B) adding potassium acetate to neutralize the SDS,

(C) adding a water insoluble protein precipitating agent ProCipitate™,

(D) isolating a liquid phase,

(E) treating the liquid phase with fumed alumina, titania, or zirconia in a dry powdered form, or in a suspension of the metallic oxides in a salt solution with weak charge attenuating properties,

(F) separating the supernatant,

(G) washing DNA-metallic oxide complex with water

(H) dissociating the nucleic acid from the DNA-metallic oxide complex under mild alkali conditions, and

(I) recovering and neutralizing the DNA containing solution.

19. A method for isolating plasmids from bacterial lysates, comprising

(A) lysing the bacteria with sodium dodecylsulfate (SDS) in alkaline solution containing a metal chelator,

(B) adding potassium acetate to neutralize the SDS,

(C) isolating a liquid phase,

(D) treating the liquid phase with fumed alumina, titania, or zirconia in a dry powdered form, or in a suspension of the metallic oxides in a salt solution with weak charge attenuating properties,

(E) separating the supernatant,

(F) washing DNA-metallic oxide complex with water

(G) dissociating the nucleic acid from the DNA-metallic oxide complex under mild alkali conditions, and

(H) recovering and neutralizing the DNA containing solution.

20. The method of claim 19, wherein the plasmids are bacterial artificial chromosomes (BAC).

21. A fumed metallic oxide configuration consisting of non-attenuated charges prepared by dispersing fumed metallic oxide particles in deionized water which has a high binding capacity for ribonucleic acid (RNA) and a marginal binding capacity for DNA.

22. A product for removing RNA from DNA containing preparations consisting of the fumed metallic oxide configuration of claim 21.

23. A method for removing contaminating RNA and DNA in the processing of recombinant proteins, comprising

adding dry fumed metallic oxide powders or suspensions of these oxides in a solution of a metal salt with weak charge attenuating properties to the proteinaceous solution.

24. A configuration of fumed metallic oxides bound to polyethylene glycol which has a higher particle density and less cross reactivity with proteins than the polyethylene glycol free fumed metallic oxides.

25. A method of binding DNA and RNA to a metallic oxide surface, comprising

adding metallic oxide to a solution of nucleic acids in a dry powdered form, and attenuating rapidly developing positive charges on metallic oxide as soon as the metallic oxide strikes the solution of the negatively charged nucleic acids.

26. A method for binding RNA but not DNA contained in a salt free medium to a metallic oxide surface in a salt free medium, comprising severe electrostatic repulsions between fully charged metallic oxide surface would prevent the large

DNA molecule from bridging two or more oxide particles and hence prevent its binding to the surface. RNA being a much smaller molecule is not subject to these constraints.

27. The method of claim – wherein the charge attenuation of the metallic oxide surface by a salt is largely dependent on the chemical nature of the atoms that constitute the ionic species and charge attenuation increases with increasing electronegativity of the atom, that constitutes the ionic species.

28. A kit for use in a method for isolating deoxyribonucleic acid (DNA) from other substances in solution, comprising

(A) a substrate for holding a specimen
(B) a chaotrope
(C) a protein precipitating agent
(D) an adsorbent comprising a fumed metallic oxide
(E) a DNA dissociating agent

29. The kit of claim 28, wherein the chaotrope is guanidine thicyanate.

30. The kit of claim 28, wherein the chaotrope is guanidine thicyanate and a buffer.

31. The kit of claim 28, wherein the protein precipitating agent is Procipitate™.

32. The kit of claim 28, wherein the DNA dissociating agent is a 0.02 molar solution of sodium hydroxide.

33. The kit of claim 28, wherein the adsorbent is a fumed metallic oxide selected from the group consisting of aluminum oxide, titanium oxide, and zirconium oxide.

34. The kit of claim 28, wherein the fumed metallic oxide is in combination with an aqueous solution of sodium salts of an anion selected from the group consisting of acetate, bicarbonate, sulfate, sulfite, chloride, oxalate, and citrate.

35. The kit of claim 28, wherein the fumed metallic oxide is in combination with 0.15M NaCl.

36. The kit of claim 28, wherein the fumed metallic oxide is in combination with deionized water.

37. The kit of claim 28, wherein the dissociating agent is selected from the group consisting of 0.02M PO_4^{2-}, 0.02M B_4O^{2-} and OH^{1-}.

38. The kit of claim 28, wherein the dissociating agent is sodium hydroxide.

39. The kit of claim 28, wherein the kit further comprises a neutralizing buffer.

40. The kit of claim 39, wherein the neutralizing buffer is TrisHCl in deionized water.

41. The kit of claim 28, wherein the adsorbent is fumed metallic oxide.

42. The kit of claim 28, wherein the adsorbent is fumed metallic oxide and is attached to a solid-phase carrier.

43. The kit of claim 41, wherein the solid-phase carrier is a plastic bead.

42. The kit of claim 41, wherein the solid-phase carrier is a microtiter plate.

43. The kit of claim 41, wherein the solid-phase carrier is solid-phase carrier of insoluble polymers.

44. The kit of claim 28, wherein the solid-phase carrier is a polymer of styrene or vinyl chloride or a copolymer thereof.

45. The method of claim 28, wherein said solid phase substrate is a stick or bead.

Figure 1

Diagramatic representation of the permenently charged fumed metaliic oxide particles dispersed in ion free water

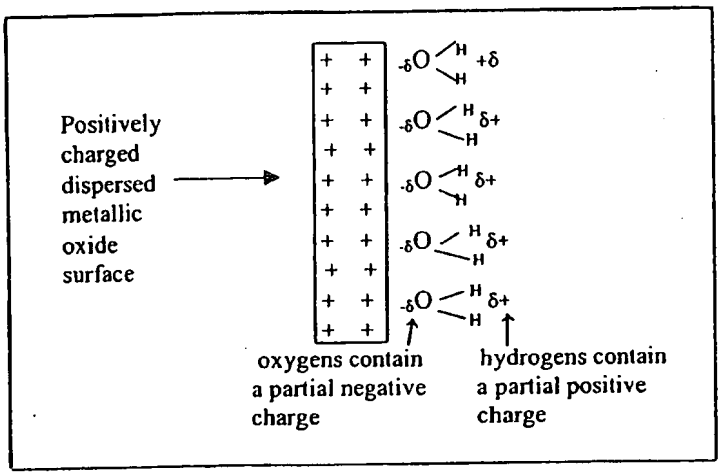


Figure 2

Diagramatic representation of attenuated charges on fumed metallic oxide particles dispersed in a sodium chloride solution.

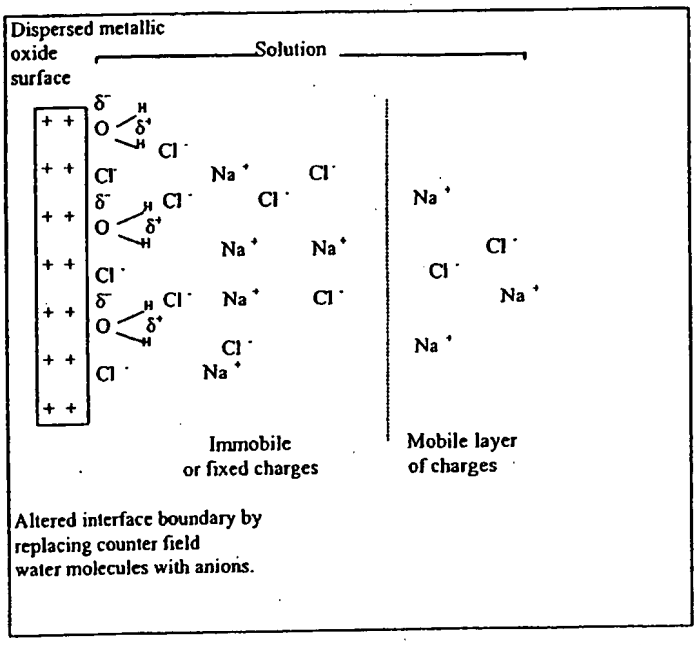
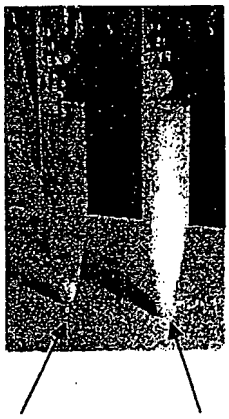


Figure 3

Aggregation and sedimentation profiles of fumed metallic oxides in the presence and absence of DNA

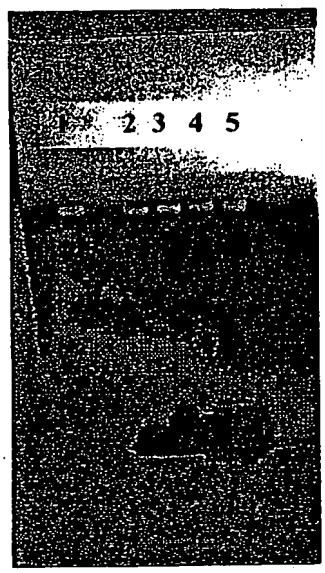


Urine spiked with White blood cells in 3M GuSCN with added dispersed metallic oxide

Urine spiked with white blood cells in the absence of 3M GuSCN with metallic oxide

Figure 4

Electrophoretic patterns of DNA isolated from whole mouse blood and from urine spiked with white blood cells using fumed Titanium Oxide P25.



Lane 1= 23 kb DNA marker

Lane 2= Mouse blood DNA disoociated with 3M guanidine thiocyanate, 100mM EDTA in 0.1M sodium acetate pH 7.0 at room temperature and treated with ProCipitate™.

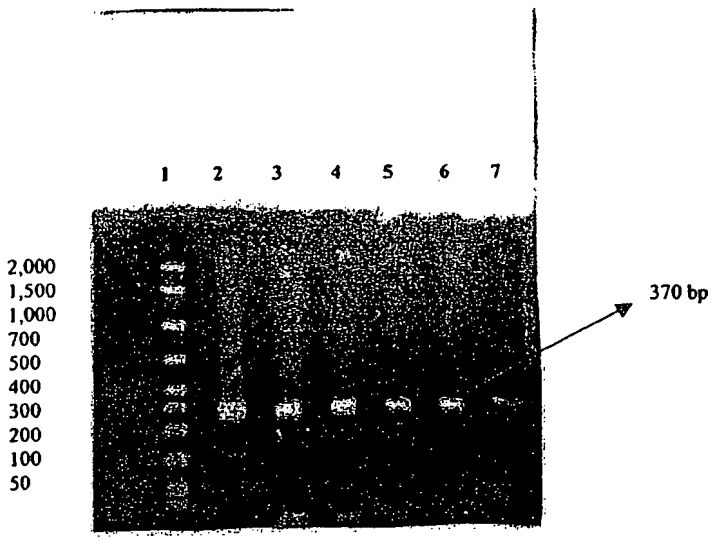
Lane 3= Mouse blood DNA dissociated with 3M guanidine thiocyanate in 0.1M sodium acetate pH 7.0 at 65°C and treated with ProCipitate™.

Lane 4= DNA isolated from urine spiked with white blood cells.

Lane 5= DNA isolated from urine spiked with white blood cells and hemoglobin and treated with ProCipitate™.

Figure 5

Polymerase Chain Reaction (PCR) patterns of DNA isolated from whole mouse blood and from urine spiked with white blood cells using fumed Titanium Oxide P25.



Lane 1= 50-2,000 bp DNA Ladder.

Lane 2= Mouse Histone H^3 amplicon 370 bp.

Lane 3= Mouse blood DNA disoociated with 3M guanidine thiocyanate, 100mM EDTA in 0.1M sodium acetate pH 7.0 at room temperature and treated with ProCipitate™.

Lane 4= Mouse blood DNA dissociated with 3M guanidine thiocyanate in 0.1M sodium acetate pH 7.0 at 65°C and treated with ProCipitate™.

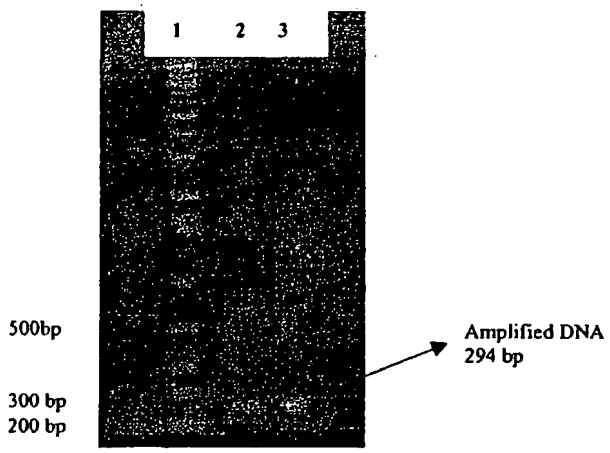
Lane 5= DNA isolated from urine spiked with white blood cells.

Lane 6= DNA isolated from urine spiked with white blood cells and hemoglobin and treated with ProCipitate™.

Lane 7= Mouse white blood cells dissociated with sodium dodecyl sulfate-EDTA and purified by the ProCipitate™/DNAble procedure.

Figure 6

Polymerase Chain Reaction (PCR) of low copy number DNA in plasma which was captured by fumed Titanium Oxide P-25 and subsequently released



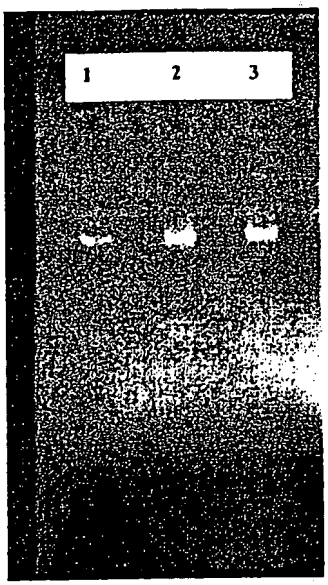
Lane 1= DNA Ladder

Lane 2= Control: Human ß actin amplicon 294 bp.

Lane 3= Amplified DNA present in low copy number in plasma 294 bp.

Figure 7

Electrophoretic Profiles of Bacterial Artificial Chromosome (BAC)



Lane 1= 25 kilobase genomic DNA marker.

Lane 2= BAC DNA isolated by the fumed titanium oxide procedure.

Lane 3= BAC DNA isolated by the alcohol-precipitation and centrifugation method.

Isolation of DNA using DNAble in a 96-well format

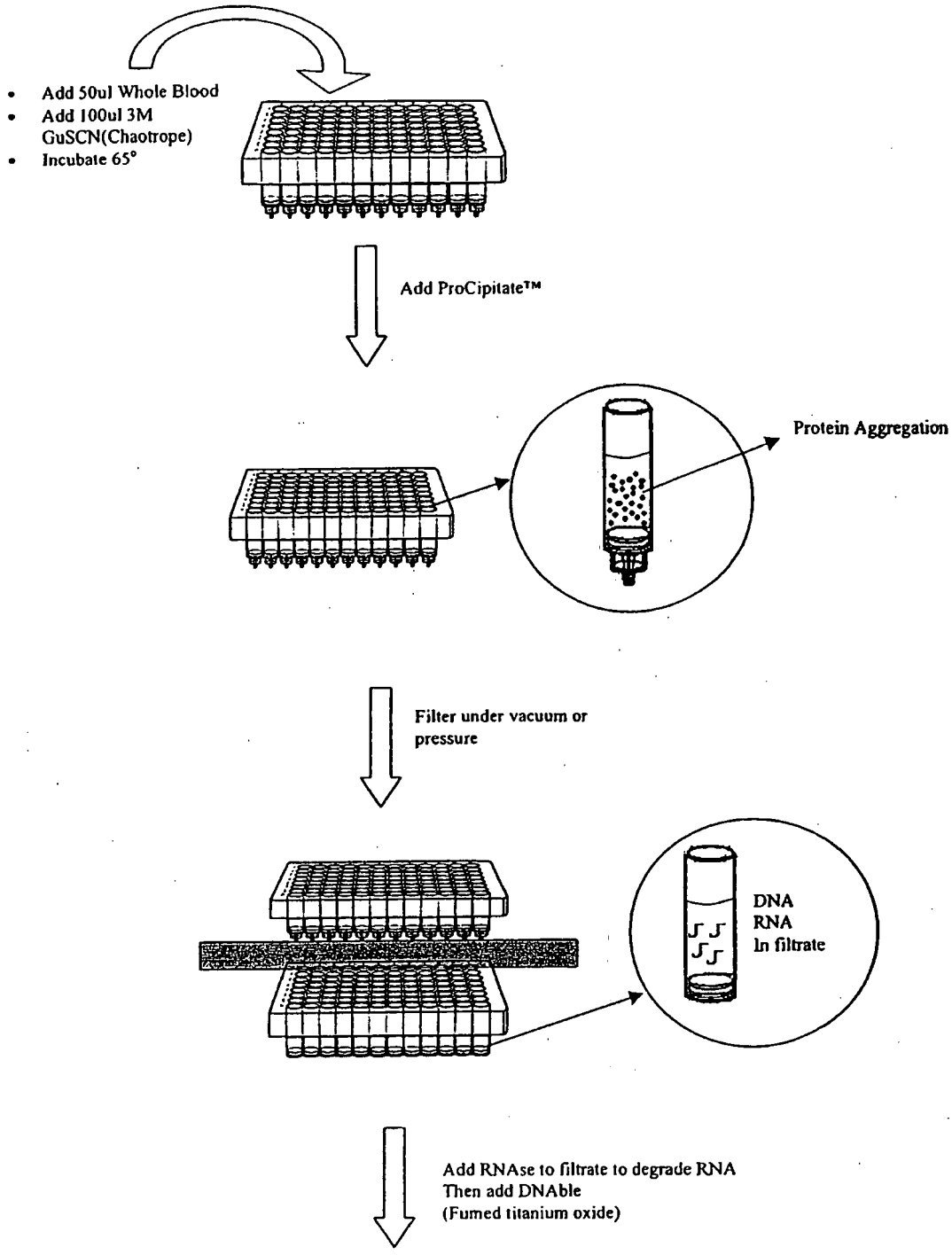


FIG. 8A

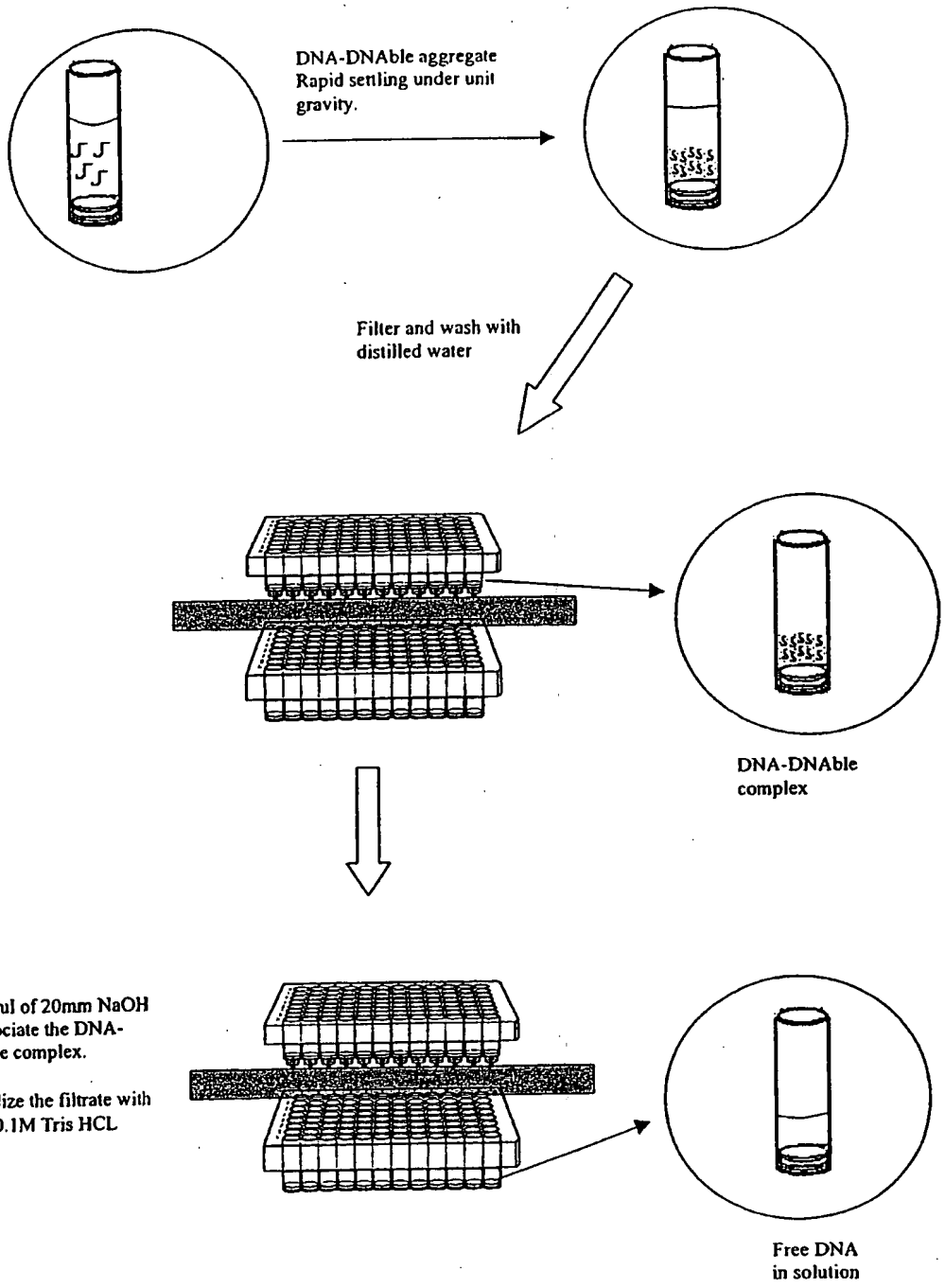

DNA-DNAble aggregate
Rapid settling under unit gravity.
Filter and wash with distilled water
DNA-DNAble complex
Add 40ul of 20mm NaOH to dissociate the DNA-DNAble complex.
Filter.
Neutralize the filtrate with 5ul of 0.1M Tris HCL
Free DNA in solution

FIG. 8B

Schematic Diagram of the Genomic 96 Experimental Protocol

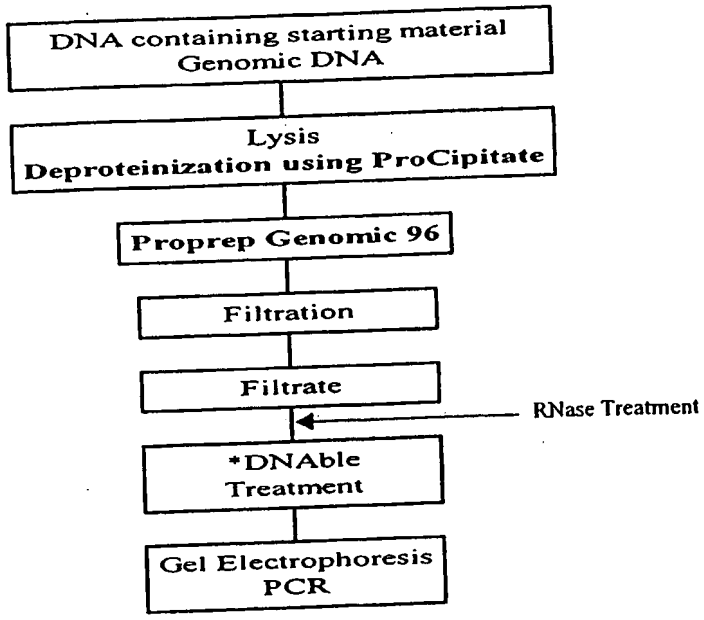


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31005

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C12Q 1/68
US CL :435/6, 91.1, 270
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.1, 270

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, MEDLINE, CAPLUS, BIOTECHNO, EMBASE, SCISEARCH, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/18514 A1 (MINNESOTA MINING AND MANUFACTURING COMPANY) 29 October 1992, see entire document.	1-26 and 28-43(pages 45-53), 42-45(page 54)
Y	US 5,234,809 A (BOOM et al) 10 August 1993, see entire document.	1-26 and 28-43(pages 45-53), 42-45(page 54)
Y	US 5,057,426 A (HENCO et al) 15 October 1991, see entire document.	1-26 and 28-43(pages 45-53), 42-45(page 54)

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
27 DECEMBER 2000	05 FEB 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	SEAN MCGARRY
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

TERRY J. DEY
PARALEGAL SPECIALIST
TECHNOLOGY CENTER 1600

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31005

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.